

AWARD NUMBER: W81XWH-13-1-0430

TITLE: Optimal Treatment of Malignant Long Bone Fracture: Influence of Method of Repair and External Beam Irradiation on the Pathway and Efficacy of Fracture Healing

PRINCIPAL INVESTIGATOR: Vincent D. Pellegrini, Jr., MD

CONTRACTING ORGANIZATION: Medical University of South Carolina
Charleston SC, 29425

REPORT DATE: October 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2014		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2013 - 29 Sep 2014	
4. TITLE AND SUBTITLE Optimal Treatment of Malignant Long Bone Fracture: Influence of Method of Repair and External Beam Irradiation on the Pathway and Efficacy of Fracture Healing				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0430	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Vincent D. Pellegrini, Jr., MD E. Lex Hanna, MD E-Mail: pelleqvd@musc.edu ; hannae@musc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Medical University of South Carolina 179 Ashley Ave Charleston SC 29425-8908				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this project is to characterize the differential effects of radiation on the two pathways of fracture healing in an established animal model of bilateral femur fracture repair. Since relocation to MUSC, the PI has been fully engaged in securing suitable facilities, collaborations, and related institutional regulatory approvals to implement the animal model at MUSC. This was accomplished in August, 2013. Surgical procedures consisting of bilateral femur fracture and repair have been completed on both cohorts of 18 animals in the year 1 SOW; all animals were imaged and have been sacrificed according to protocol schedule. Group I specimens are being analyzed with micro CT to assess callus volume and character. Group II specimens are being processed for RNA isolation and PCR array analysis. Year 2 SOW animal procedures in Group III will begin on schedule in the fifth quarter of the award.					
15. SUBJECT TERMS Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Overall Project Summary.....	4
4. Key Research Accomplishments.....	4
5. Conclusion.....	5
6. Publications, Abstracts, and Presentations.....	5
7. Inventions, Patents and Licenses.....	5
8. Reportable Outcomes.....	5
9. Other Achievements.....	5
10. References.....	5
11. Appendices.....	6

Introduction:

We propose that there exists an optimal pathway of fracture healing that is relatively less impaired by the effects of radiation therapy, and that this pathway would logically be preferentially exploited in the treatment of pathologic fractures requiring radiation for local tumor control. Further, to the extent that the method of surgical fracture fixation dictates the pathway of fracture healing, we propose that there will likewise exist an optimal method of surgical fracture repair based on the induced biology of fracture healing that would logically be utilized in the setting of malignant skeletal disease where it is anticipated that radiation would be employed as postoperative adjunctive therapy. Accordingly, we will investigate the differential effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant osteoporotic fractures that require external beam irradiation for local tumor control.

Keywords:

Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model

Overall Project Summary:

*Current objectives: **Bilateral femur fracture procedures have been completed on 36 study animals (18 in Group I and 18 in Group II) as outlined in Quarters 2 and 3 under Task***

1. All animals have been survived out to predetermined time points, euthanized, and bilateral hindlimb tissue harvest has been accomplished producing **a total**

of 72 specimens. Thirty-six specimens from Group I are currently undergoing analysis by micro CT for histomorphometry as well as immunohistology to evaluate callus formation. The thirty-six specimens from Group II are being processed for RNA expression and PCR analysis to evaluate differential signaling of bone healing.



Figure 1. AP and lateral plain film radiographs of Sprague-Dawley rat post bilateral femur fracture procedure.

Results: We are currently analyzing harvested specimens from the first 36 study animals by micro CT imaging and RNA analysis to identify differences in fracture healing via the two pathways. *Specimens collected from Group I animals for RNA analysis have been held in order to facilitate batch processing. Analysis of these samples is currently underway.* Group II specimens are being processed for histomorphometry and immunohistology. **Micro CT data acquired from Group II specimens during the last two months are included in supplemental appendices.**

Progress and Accomplishments: Facilities have been established for conduct of the bilateral femur model at MUSC and all necessary collaborations and institutional regulatory approvals have been obtained. We have completed bilateral surgical femur fracture procedures on all 36 animals indicated in Task 1 in the SOW for the first year of the study. Those harvested specimens are currently being processed per protocol as delineated under Tasks 2 and 3. The project is currently on schedule and Task 1 work specified for the 5th quarter will occur as planned. We do not anticipate any delays affecting the study in the near future.

Key Research Accomplishments: Animal experiments completed on schedule and processing underway with data becoming available on a rolling basis according to tissue processing needs and constraints.

Conclusion: Research work on schedule as proposed and planned. No results to report.

Publications, Abstracts, and Presentations: Nothing to report.

Inventions, Patents and Licenses: Nothing to report.

Reportable Outcomes: Nothing to report.

Other Achievements: The experience and training provided by this award during the past year directly contributed to the hiring of the past research resident to a position in the orthopaedic residency at the Medical University of South Carolina.

References: None.

Appendices:

1. Micro CT Data – Group II animals.

Sample Information				Callus volume (CV)		Bone Volume (BV)		CV/BV			EX/IN	Tissue mineral density (TMD)		Tissue mineral density (TM D)		Notes
Group	Housing time	Number		External (mm ³)	Internal (mm ³)	Bone: (mm ³)	True: (mm ³)	External	Internal	Total		External (mg HA/ccm)	Internal (mg HA/ccm)	Bone: (mg HA/ccm)	True: (mg HA/ccm)	
Control	Plate	1 wk	12	3.64	5.83	79.19	73.36	0.05	0.08	0.13	0.62	762.78	1004.73	1201.68	1217.33	3D reconstruction done.
Control	Plate	1 wk	19	5.70	1.96	82.11	80.15	0.07	0.02	0.10	2.91	839.72	880.56	1198.91	1206.69	
Control	Plate	1 wk	20	3.72	2.32	71.55	69.23	0.05	0.03	0.09	1.60	743.24	846.85	1206.04	1218.08	
			Ave	4.35	3.37	77.62	74.25	0.06	0.05	0.10	1.71	781.91	910.71	1202.21	1214.03	
			St dev	1.17	2.14	5.45	5.51	0.01	0.03	0.02	1.15	51.01	83.15	3.59	6.37	
Control	Plate	2 wk	13	17.87	4.30	80.51	76.21	0.23	0.06	0.29	4.16	855.74	853.43	1219.00	1239.63	3D reconstruction done.
Control	Plate	2 wk	17	10.33	3.04	80.29	77.25	0.13	0.04	0.17	3.40	818.52	766.20	1217.70	1235.47	
Control	Plate	2 wk	18	13.69	5.21	78.90	73.69	0.19	0.07	0.26	2.63	786.48	908.99	1192.61	1212.66	
			Ave	13.96	4.18	79.90	75.72	0.18	0.06	0.24	3.39	820.25	842.87	1209.77	1229.25	
			St dev	3.78	1.09	0.87	1.83	0.05	0.02	0.06	0.76	34.66	71.98	14.88	14.52	
Control	Plate	4 wk	6	13.02	3.61	70.05	66.44	0.20	0.05	0.25	3.61	882.60	879.54	1069.55	1079.87	3D reconstruction done.
Control	Plate	4 wk	7	22.43	8.22	83.59	75.37	0.30	0.11	0.41	2.73	894.54	857.78	1125.01	1154.15	
Control	Plate	4 wk	16	15.58	3.50	77.99	74.49	0.21	0.05	0.26	4.45	881.86	888.06	1208.17	1223.21	
			Ave	17.01	5.11	77.21	72.10	0.23	0.07	0.30	3.60	886.33	875.13	1134.24	1152.41	
			St dev	4.87	2.69	6.80	4.92	0.06	0.03	0.09	0.86	7.12	15.61	69.77	71.68	
Control	Nail	1 wk	12	4.70	2.66	89.42	86.76	0.05	0.03	0.08	1.77	804.72	838.43	1234.37	1246.51	3D reconstruction done.
Control	Nail	1 wk	19	3.72	1.53	91.56	90.03	0.04	0.02	0.06	2.43	732.50	863.15	1218.44	1224.48	
Control	Nail	1 wk	20	1.99	2.62	88.37	85.75	0.02	0.03	0.05	0.76	716.66	942.97	1219.00	1227.43	
			Ave	3.47	2.27	89.78	87.51	0.04	0.03	0.07	1.65	751.29	881.52	1223.94	1232.81	
			St dev	1.37	0.64	1.63	2.24	0.02	0.01	0.02	0.84	46.94	54.64	9.04	11.96	
Control	Nail	2 wk	13	13.57	1.67	86.87	85.20	0.16	0.02	0.18	8.13	795.46	885.93	1215.85	1222.32	3D reconstruction done. <i>Nail broken inside.</i>
Control	Nail	2 wk	17	6.24	1.12	87.19	86.07	0.07	0.01	0.09	5.57	734.63	793.52	1230.20	1235.88	
Control	Nail	2 wk	18	9.69	2.09	85.88	83.79	0.12	0.02	0.14	4.64	793.43	875.93	1221.96	1230.59	
			Ave	9.83	1.63	86.65	85.02	0.12	0.02	0.13	6.11	774.51	851.79	1222.67	1229.60	
			St dev	3.67	0.49	0.68	1.15	0.04	0.01	0.05	1.81	34.55	50.71	7.20	6.84	
Control	Nail	4 wk	6	37.36	1.75	72.14	70.39	0.53	0.02	0.56	21.35	850.10	776.11	1154.18	1163.58	3D reconstruction done.
Control	Nail	4 wk	7	41.25	2.28	87.16	84.88	0.49	0.03	0.51	18.09	820.46	943.43	1139.27	1143.27	
Control	Nail	4 wk	16	34.73	1.90	82.96	81.06	0.43	0.02	0.45	18.28	824.91	912.41	1190.02	1196.53	
			Ave	37.78	1.98	80.75	78.78	0.48	0.03	0.51	19.24	831.82	877.32	1161.16	1168.21	
			St dev	3.28	0.27	7.75	7.51	0.05	0.00	0.05	1.83	15.98	89.01	26.08	26.31	
X-Ray	Plate	1 wk	1	7.81	2.77	80.65	77.88	0.10	0.04	0.14	2.82	701.11	740.00	1070.75	1082.51	3D reconstruction done.
X-Ray	Plate	1 wk	2	2.81	3.18	71.35	68.17	0.04	0.05	0.09	0.88	725.00	712.50	1078.99	1096.09	
X-Ray	Plate	1 wk	8	5.78	2.43	81.59	79.16	0.07	0.03	0.10	2.38	881.76	742.50	1201.96	1216.06	
			Ave	5.47	2.79	77.86	75.07	0.07	0.04	0.11	2.03	769.29	731.67	1117.23	1131.55	
			St dev	2.51	0.38	5.66	6.01	0.03	0.01	0.02	1.01	98.13	16.65	73.49	73.50	
X-Ray	Plate	2 wk	3	8.34	15.40	99.33	83.93	0.10	0.18	0.28	0.54	781.02	787.22	1065.48	1116.54	3D reconstruction done.
X-Ray	Plate	2 wk	9	1.10	3.27	73.46	70.19	0.02	0.05	0.06	0.34	821.95	871.39	1268.72	1287.23	
X-Ray	Plate	2 wk	15	8.99	2.91	84.41	81.50	0.11	0.04	0.15	3.09	839.08	715.09	1182.80	1199.50	
			Ave	6.14	7.19	85.73	78.54	0.08	0.09	0.16	1.32	814.02	791.23	1172.33	1201.09	
			St dev	4.38	7.11	12.99	7.33	0.05	0.08	0.11	1.53	29.83	78.23	102.02	85.36	
X-Ray	Plate	4 wk	10	5.15	15.20	98.70	83.50	0.06	0.18	0.24	0.34	940.38	899.36	1123.07	1163.79	3D reconstruction done.
X-Ray	Plate	4 wk	11	12.30	1.58	78.79	77.21	0.16	0.02	0.18	7.78	927.41	746.39	1189.93	1199.01	
X-Ray	Plate	4 wk	14	14.07	10.90	79.25	68.35	0.21	0.16	0.37	1.29	930.56	851.21	1162.42	1212.05	
			Ave	10.51	9.23	85.58	76.35	0.14	0.12	0.26	3.14	932.78	832.32	1158.47	1191.62	
			St dev	4.72	6.96	11.36	7.61	0.07	0.09	0.09	4.05	6.76	78.21	33.60	24.96	
X-Ray	Nail	1 wk	1	4.79	3.55	84.17	80.62	0.06	0.04	0.10	1.35	718.52	778.70	1198.72	1217.22	3D reconstruction done.
X-Ray	Nail	1 wk	2	4.44	0.80	81.86	81.06	0.05	0.01	0.06	5.55	779.44	806.02	1144.09	1147.43	
X-Ray	Nail	1 wk	8	6.49	2.00	96.42	94.42	0.07	0.02	0.09	3.25	816.39	903.15	1234.00	1241.01	
			Ave	5.24	2.12	87.48	85.37	0.06	0.03	0.09	3.38	771.45	829.29	1192.27	1201.88	
			St dev	1.10	1.38	7.83	7.84	0.01	0.02	0.02	2.10	49.42	65.41	45.30	48.64	
X-Ray	Nail	2 wk	3	8.66	3.37	89.23	85.86	0.10	0.04	0.14	2.57	819.72	923.15	1156.50	1165.66	3D reconstruction done. <i>Nail broken inside.</i>
X-Ray	Nail	2 wk	9	4.27	0.76	72.29	71.53	0.06	0.01	0.07	5.62	808.98	869.26	1271.22	1275.49	
X-Ray	Nail	2 wk	15	15.72	2.68	82.74	80.06	0.20	0.03	0.23	5.87	772.68	699.16	1217.15	1234.49	
			Ave	9.55	2.27	81.42	79.15	0.12	0.03	0.15	4.68	800.46	830.52	1214.96	1225.21	
			St dev	5.78	1.35	8.55	7.21	0.07	0.02	0.08	1.84	24.65	116.91	57.39	55.50	
X-Ray	Nail	4 wk	10	34.07	5.87	68.22	62.35	0.55	0.09	0.64	5.80	884.36	853.34	1132.05	1158.29	3D reconstruction done.
X-Ray	Nail	4 wk	11	31.36	1.98	104.60	102.62	0.31	0.02	0.32	15.84	841.48	926.30	1204.09	1209.45	
X-Ray	Nail	4 wk	14	22.12	0.61	83.60	82.99	0.27	0.01	0.27		851.39	800.19	1196.59	1199.50	

2. 3-D micro CT reconstructions comparing callus volume of femora healing via plate fixation versus IM nail fixation.

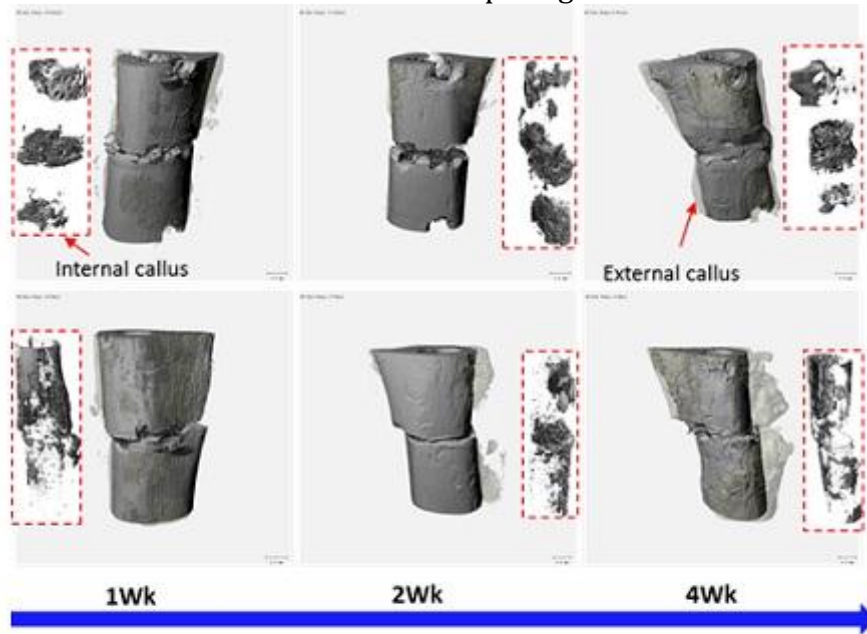


Figure 2. 3-D CT reconstruction of femora treated with IM nail or plate fixation at 1, 2 and 4 weeks. External callus is represented by the light grey portion surrounding the darker bone. Internal callus is represented in the outlined box.